# Ultralow Oxygen Treatment for Postharvest Control of *Nasonovia ribisnigri* (Homoptera: Aphididae) on Iceberg Lettuce

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ABSTRACT The aphid *Nasonovia ribisnigri* (Mosley) is a common pest of lettuce in the United States. It hinders export of U.S. lettuce to the overseas market such as Japan where it is a quarantined pest. Ultralow oxygen treatments were studied for control of the insect on iceberg lettuce. Small-scale ultralow oxygen treatments in plastic jars were conducted at 1, 5, and 10°C for different durations to determine effective treatment against nymphs and alates of *N. ribisnigri*. At oxygen levels of 0.015–0.025%, *N. ribisnigri* can be controlled in 3 d at 1°C, 2 d at 5°C, and 1 d at 10°C. Large-scale ultralow oxygen treatments were conducted in bulk container treatment chambers with commercial iceberg lettuce heads for 2 d at 6°C with oxygen levels of 0.015 and 0.025% and for 3 d at 3°C with oxygen level of 0.015%. All treatments achieved complete control of *N. ribisnigri*. No negative impact on lettuce quality was detected after 2 wk of posttreatment storage. Therefore, the selected treatments have potential to be commercially developed for postharvest control of *N. ribisnigri* on iceberg lettuce.

KEY WORDS ultralow oxygen, postharvest, quarantine treatment, aphid, lettuce

POSTHARVEST INSECT CONTROL IS a major challenge in the export of U.S. lettuce to overseas markets such as Japan. The interceptions of live insects that are of phytosanitary concern by inspectors in overseas markets invariably lead to rejection or fumigation of lettuce shipments. Nasonovia ribisnigri (Mosley) (Homoptera: Aphididae) is a major lettuce pest in the United States. (Chaney 1999, Palumbo 2000, Palumbo and Hannan 2002, Liu 2004) and is a quarantined pest in Japan and some other countries. Fumigation treatment with methyl bromide or hydrogen cyanide for postharvest insect control causes injuries and reduced shelf life of lettuce. Because no safe fumigants currently exist for treating insects on harvested lettuce, an alternative is needed that is both efficacious in controlling insects and safe to lettuce.

Controlled atmosphere (CA) is one of the potential alternatives that has been studied for postharvest control of insects on perishable agricultural commodities. including lettuce (Ke and Kader 1992, Whiting et al. 1992, Carpenter and Potter 1994, Mitcham et al. 2001, Liu 2003). CA treatments usually use elevated CO<sub>2</sub>. Iceberg lettuce, however, is very susceptible to injury by  $CO_2$  (Lipton et al. 1972; Brecht et al. 1973a, b, c; Stewart and Uota 1976). Recommendations suggest storing iceberg lettuce under 1-3% O<sub>2</sub> and 0% CO<sub>2</sub> (Beaudry 2000). CO<sub>2</sub> levels as low as 1% can cause injury to susceptible iceberg lettuce (Lipton et al. 1972). Elevated CO<sub>2</sub> levels cause brown stains in the form of brown lesions on or near the midribs of lettuce leaves. The time required to kill insects often exceeds the time that lettuce can tolerate (Ke and Kader 1992, Mitcham et al. 2001).

Atmosphere with  $\leq 1\%$  O<sub>2</sub> is referred as ultralow oxygen (ULO). Limited studies show that ULO treatments can kill insects, inhibit microbial growth, or extend the shelf life of fresh commodities (Zheng et al. 1993, Mitcham et al. 2001, Shellie 2002, Legnani et al. 2004). However, low O<sub>2</sub> levels also can cause injury to lettuce (Lipton 1967; Lipton et al. 1972; Cantwell et al. 1995, 1996; Mitcham et al. 2001). Low  $O_2$  injuries occur in the form of gray dead patches on wrapper leaves and as a brownish discoloration on heart leaves (Lipton et al. 1972). Storage under 0.25% O<sub>2</sub> at 2°C for 7 d causes injury to iceberg lettuce (Lipton 1967, 1971; Lipton et al. 1972). CA with  $O_2$  level of 0.02% was found to kill aphids in 7-21 d at 0°C (Zheng et al. 1993, Mitcham et al. 2001). However, lettuce also was reported to tolerate 0.02% O<sub>2</sub> CA for 13 d at 0°C (Cantwell et al. 1995, 1996; Mitcham et al. 2001).

Although previous studies did not yield promising treatments for postharvest insect control, the studies were very limited and more studies are warranted to fully explore the potential of CA for postharvest insect control on fresh commodities. In the current study, ULO treatments were tested against *N. ribisnigri* at different temperatures to determine effective treatments for insect control and potential impact on postharvest quality of iceberg lettuce.

# **Materials and Methods**

Insects. N. ribisnigri colonies were established from field-collected insects in 2001 and were reared on lettuce plants in screen cages in a greenhouse. N. ribisnigri newly collected in 2003 were added to the

colonies. For each test, apterous N. ribisnigri were placed on lettuce leaf pieces in plastic petri dishes (5.5 cm in diameter,  $\approx 15$  aphids per petri dish), and the petri dish tops were secured to the bottoms with tape. The petri dishes had narrow spaces between the tops and the bottoms that permit ventilation. Alates were collected in plastic vials (2.5 cm in diameter by 7 cm in height, 10-15 alates per vial) containing a piece of lettuce leaf by using a vacuum-powered aspirator. The vials were sealed with lids with screened windows for ventilation.

Effects of Small-Scale Ultralow Oxygen Treatments on Insect Survival. Ultralow oxygen conditions were established in large drum chambers filled with lettuce by intermittently releasing  $N_2$  and air into the chambers. The ultralow oxygen atmosphere was circulated using an air pump through a series of plastic jars with insects for desired time intervals to accomplish ultralow oxygen treatments.

Commercial iceberg lettuce heads from supermarkets were placed in large treatment chambers modified from metal drums (76 liters). A paper bag with 100 g of Sodasorb (Grace & Co., Atlanta, GA) was added in each drum to absorb carbon dioxide. A 12-V d.c. circulation fan was set up in each drum to mix air constantly. The drums were sealed with lids and boltrings and placed in refrigerators that were equipped with external temperature controllers to set precise temperatures and 12-V d.c. fans inside to circulate air constantly. Nitrogen gas with  $\approx 0.1\%$  O<sub>2</sub> was supplied from a nitrogen generator (Balston 75-7820, Parker Hannifin Co., Tewksbury, MA) and stored in a compression tank. The nitrogen was released into the metal drum chambers through the inlets on the lids, and the inlets were extended to the bottom of the drum chambers. The outlets of the chambers were piped into a 32-cm column of water to maintain ≈3.6 kPa (0.5 psi) positive pressure in the drum chambers. Each lid had a third port linked to an oxygen analyzer (Series 800, IL Instruments, Inc., Johnsburg, IL).

Flowmeters were used to regulate flow rates of nitrogen to the drums. Solenoid valves controlled by programmable timers (ChronTrol XT, ChronTrol Corp., San Diego, CA) were used to control flow of nitrogen to the drums and flows of air from drums to the oxygen analyzer. Nitrogen was released to drums intermittently (10-20 s every 10 min, depending on treatments). Respiration of lettuce reduced oxygen levels in the treatment chambers. Oxygen levels for treatments were controlled by adding air into the nitrogen gas stream once the oxygen level in a drum fell below a set threshold and triggered the alarm relay of the oxygen analyzer. Desired ultralow oxygen CA was maintained by a combination of intermittent releasing of nitrogen with low level of oxygen into the treatment drums, consumption of oxygen by lettuce, and adding air based on feedback control of the oxygen analyzer. Carbon dioxide levels in the drums were monitored periodically with a carbon dioxide analyzer (model 302M, Nova Analytical Systems, Inc., Niagara Falls, NY) and were kept below 0.2%.

To conduct small-scale ultralow oxygen treatments against *N. ribisnigri*, one or more plastic jars ( $\approx$ 1 liter) with insects and an air pump (SP6000, Smart Products, Inc., Morgan Hill, CA) were linked in series with nylon tubing (1.6 mm i.d.) and linked to the inlet and outlet ports of a drum chamber. Each jar had two to three petri dishes with N. ribisnigri nymphs and two vials with *N. ribisnigri* alates together with one vial with a moist paper towel for treatment. The jars were sealed with lids. Each lid had two ports. For treatments with different durations, several jars were linked in a series. The air pump was linked to the one end of the jar series and circulated air from the drum with desired oxygen level through the jars. The treatments were started by purging air out of jars with nitrogen for at least 10 min and then connecting the jars and the air pump to the large drum. This allowed establishment of desired ultralow oxygen levels quickly in the jars for the treatments. To end a treatment at different time intervals, a jar was disconnected from the series and the tubing was quickly reconnected to allow air circulation while the air pump was turned off. The positive air pressures in the drums prevented outside air from getting into the system.

Four drum chambers were set up in two refrigerators to conduct ULO treatments. Two oxygen levels, 0.015 and 0.025%, were tested simultaneously at two different temperatures. Three temperatures, 1, 5, and  $10^{\circ}$ C with variation of  $\pm 0.5^{\circ}$ C, were used in this study. ULO treatments at 1 and 5°C lasted for 1, 2, and 3 d, respectively, and ULO treatments at 10°C lasted only 1 d. Treatment was replicated two to three times for any combination of treatment time, temperature, and oxygen level. Petri dishes with nymphs and plastic vials with alates were held as controls in the refrigerators in zip-lock bags with ventilation holes (four holes of 1-cm diameter for each bag) containing moist paper towels. A total of 14 treatment combinations were tested against  $\geq 2,500$  nymphs and  $\geq 1,500$  alates of N. ribisnigri in the small-scale ULO treatments.

After each treatment, jars were kept in an environmental chamber at 24°C and a photoperiod of 14:10 (L:D) h for 1 d. Insect mortality was then scored under a magnifying glass or dissecting scope. Insects that failed to move appendages in response to repeated probing with a soft brush were classified as dead. Moribund aphids that moved appendages but failed to walk normally were provided fresh leaf pieces and incubated for one more day before they were evaluated again for survivorship.

Effects of Ultralow Oxygen Treatment on Insect Survival and Lettuce Quality. ULO treatments were conducted in box chambers modified from large plastic bulk containers (107 by 74 by 71 cm). The containers were modified by adding sleeves of laminated aluminum oxygen barrier film to the opening and adding two ports to each of the two opposite walls of the container. Commercial iceberg lettuce ≈1 wk old or less after harvest was obtained from supermarkets. Lettuce heads were wrapped in plastic sleeves and packed in lettuce cartons (22–24 lettuce heads per lettuce carton). Lettuce heads with obvious defects

such as mechanical injury were excluded to maintain a consistent level of quality. Three cartons of lettuce were placed in each chamber. N. ribisnigri nymphs in petri dishes and alates in plastic vials were placed at various positions between lettuce heads in the lettuce cartons. A paper bag with 100 g of Sodasorb was added to each lettuce carton to absorb carbon dioxide. A Hobo temperature and humidity sensor was placed in one of lettuce cartons in each chamber. A 12-V d.c. fan was installed in each chamber to circulate air in the chamber. The chambers were sealed by folding the sleeve of each chamber around a metal plate and fastening the sleeve between two metal plates with binders. The containers were covered with lids, and the lids were further secured to the containers with wood boards and metal chains. Each treatment chamber had ports for inflow of nitrogen gas and outlet of the air from the chamber. The outlet tubing had the end immersed in water to allow air in the chamber to dissipate as air pressure in the chamber increased and prevent outside air from leaking into the chamber. An air pump was linked to two ports on opposite walls of the container to circulate air in the chamber. The outlet of the air pump was branched to a solenoid valve and led to the oxygen analyzer.

Two- and 3-d ULO treatments were conducted at 6 and 3°C, respectively. Temperature varied within  $\pm 0.5$ °C. Oxygen levels of 0.015 and 0.025% were used in the 2-d treatment, and oxygen level of 0.015% was used in the 3-d treatment. The treatments were started by flushing the treatment chambers with nitrogen from the nitrogen generator intermittently (2 min flushes every 5 min) to reduce oxygen levels inside the chambers until oxygen level in the chambers dropped below 0.15-0.2%. The pressure in each chamber was maintained below 2.0 kPa (0.3 psi) above the ambient pressure. Then nitrogen input rate was changed to 8-15 s flush every 5 min at 20 liters/min. The difference in air pressures in the chambers and outside was almost undetectable (below 0.6 kPa [0.1 psi]). It took  $\approx$ 8-10 h after beginning to purge the chambers for oxygen levels to drop below 0.05%. The treatment time was counted from time when oxygen levels dropped below 0.05% to the end of treatment. Therefore, the total treatment time from the beginning of the purge cycle to the end of treatment was longer than the 2or 3-d treatment duration. All treatments were replicated two times. In each test, two or three petri dishes with N. ribisnigri nymphs and two or three plastic vials with N. ribisnigri alates were put in each lettuce carton. A total of >1,100 nymphs and >800 alates were used in the large ULO treatments. Controls were put in 20 by 17.5-cm zip-lock bags with ventilation holes together with moist paper towel in the same cooler.

After ULO treatments, insects were retrieved from each lettuce carton, and lettuce heads were stored in a walk-in cooler at 2°C. Insect mortality was scored after 1-d incubation in the environmental chamber as detailed above. Lettuce quality was evaluated after 2 wk of posttreatment storage. Visual quality for marketability was evaluated using the scoring system of Kader et al. (1973) after removing the plastic wrap-

Table 1. Mortality of *N. ribisnigri* nymphs and alates in response to ultralow oxygen treatments with different oxygen levels and durations at different temperatures

т	Time (d)	O <sub>2</sub> (%)	Nyn	ph mortality	Alate mortality		
Temp (°C)			n	Mean ± SE (%)	n	Mean ± SE (%)	
1	1	0.015	114	$71.7 \pm 8.2$	73	$79.5 \pm 9.4$	
		0.025	100	$63.7 \pm 2.9$	55	$63.1 \pm 6.9$	
	2	0.015	222	$95.1 \pm 2.6$	143	$98.1 \pm 1.3$	
		0.025	167	$95.9 \pm 1.9$	101	$97.3 \pm 1.7$	
	3	0.015	213	100	110	100	
		0.025	172	$99.4 \pm 0.6$	84	$98.7 \pm 1.3$	
5	1	0.015	178	$89.9 \pm 2.4$	115	$76.1 \pm 8.0$	
		0.025	177	$92.0 \pm 2.3$	102	$95.5 \pm 5.7$	
	2	0.015	236	100	131	100	
		0.025	212	100	125	$94.4 \pm 3.1$	
	3	0.015	201	100	134	100	
		0.025	96	100	65	100	
10	1	0.015	191	$99.5 \pm 0.5$	111	100	
		0.025	169	100	114	100	

The average mortality rates of *N. ribisnigri* nymphs and alates in pooled controls were 9.9 and 11.7%, respectively.

ping sleeves. The score ranged from 1 (extremely poor) to 9 (perfect) with 3, 5, and 7 scores in between representing poor, fair, and good quality, respectively (Kader et al. 1973). Head circumferences and weights were measured. Lettuce heads were then cut into halves and further cut into smaller sections to detect any discoloration of heartleaves. Approximately 15–20 heads from each treatment were evaluated.

Data Analysis. Insect mortality data were transformed by arcsine square-root before analysis of variance (ANOVA). The Fit model platform of JMP (SAS Institute 2002) was used to analyze data. For lettuce, head density was estimated based on head weights and circumferences assuming sphere-shape for lettuce heads using the following formulas: volume =  $(4/3) \pi r^3$ , where r (radius) equals circumference/ $(2\pi)$  and  $\pi(PI)$  is 3.1415926; density = weight/volume. Head weight and density data were analyzed using ANOVA. Tukey–Kramer multiple range test was used to compare mean weight and density of lettuce heads from different sources.

## Results and Discussion

Mortality of N. ribisnigri nymphs and alates increased with increased treatment duration and temperature and complete control was achieved at all three temperatures (Table 1). For treatments at  $1^{\circ}$ C, mortality increased progressively with increased treatment time. Total control of both nymphs and alates was achieved with 0.015% ULO treatment in 3 d. At 5°C, total control of nymphs and alates was achieved with 0.015% ULO treatment in 2 d. At 10°C, 100% mortality was achieved for both nymphs and alates with 0.025% ULO treatment and for alates with 0.015% ULO treatment in 1 d (Table 1). In general, there were significant effects of temperature and treatment time on aphid mortality. There were also significant interactions between temperature and time, indicating synergistic effects between tempera-

Table 2. Effect of different factors in ultralow oxygen treatments on mortality of N. ribisnigri

	Nparm	df	Ny	mphs	Alates		
Source			F	P > F	F	P > F	
Temp	1	1	29.299	< 0.0001	10.195	0.0020	
Time	1	1	82.277	< 0.0001	48.300	< 0.0001	
$O_2$	1	1	0.936	0.3362	0.493	0.4846	
$\overline{\text{Temp}} \times \text{time}$	1	1	11.430	0.0011	6.857	0.0105	

Mortality rates were transformed by arcsine square-root analysis of variance. Nparm is the number of parameters associated with the effect. JMP Fit Model platform was used (SAS Institute 2002).

ture and time in accelerating insect mortality. There were no significant effects of oxygen level (Table 2).

All large-scale ULO treatments killed N. ribisnigri and caused no injury to lettuce heads. ULO treatments with 0.015 and 0.025% oxygen at 6°C killed all nymphs and alates of N. ribisnigri in 2 d, and ULO treatment with 0.015% oxygen at 3°C killed all nymphs and alates of N. ribisnigri in 3 d. Mortality rates for controls ranged between 4.6 and 20% (Table 3). For the 2-d treatments, a few nymphs were found moribund 1 d after the treatments. They were kept on fresh leaves in petri dishes and incubated in the environmental chamber. All of them died 2 d after the treatment and were classified as dead in calculating effects of the treatments on insect mortality. Visual quality of all treated lettuce heads was the same as control heads and the quality score of 7 (good heads with minor defects that does not affect marketability) was given to most heads with a few exceptions of score of 5 (minor injury with limited marketability mostly because of leaf browning from mechanical injuries). Dissection of lettuce heads showed no symptoms of injuries by ULO treatments. Lettuce heads from six sources (combination of different purchase dates and different supermarkets) also showed significant differences in weight and head density (Table 4). The means of head weights ranged from 566 g to >800 g, and head density ranged from 0.290 to 0.429 g/cm<sup>3</sup>, representing a wide range of head size and maturity.

The oxygen levels used in this study were selected based on preliminary ULO treatments conducted previously (unpublished data). The insignificant effects of oxygen level were probably because of the narrow difference of the two oxygen levels and total control of aphids in some combinations of temperature and treatment duration. ULO treatments with much higher levels of oxygen (0.05%) did take longer time

Table 3. Mortality of N. ribisnigri nymphs and alates in largescale ultralow oxygen treatments

Temp (°C)	Time (d)	$O_2 \ (\%)$	Nymj	phal mortality	Alate mortality		
			n	Mean ± SE (%)	n	Mean ± SE (%)	
3	3	0.015	360	100	245	100	
6	2	Control 0.015 0.025 Control	100 302 240 110	7.0 100 100 6.4	80 207 205 65	20.0 100 100 4.6	

Table 4. Weight and density of iceberg lettuce heads used in ultralow oxygen treatments for control of N. ribisnigri

Lettuce	n	Lettuce he	ead wt	Head density (g/cm <sup>3</sup> )	
source		Mean ± SE	Min	Max	Mean ± SE
A	39	761 ± 22ab	537	1098	$0.429 \pm 0.014a$
В	39	$611 \pm 20c$	382	956	$0.337 \pm 0.011$ be
C	18	$685 \pm 19 be$	550	805	$0.290 \pm 0.009c$
D	21	$783 \pm 25ab$	605	1091	$0.373 \pm 0.014b$
E	20	$839 \pm 33a$	580	1105	$0.379 \pm 0.014ab$
F	20	$566 \pm 30c$	329	778	$0.321\pm0.017bc$

The lettuce source refers to different stores and different dates where and when lettuce heads were purchased for the study. For the mean weight of lettuce heads and head density, the values followed by the same letter were not significiantly differet, Tukey–Kramer multiple range test (SAS Institute 2002).

to kill N. ribisnigri compared with treatments in the current study at the same temperature (unpublished data). In the current study, aphids were in petri dishes and plastic vials. On infested lettuce heads, N. ribisnigri distributes through lettuce heads with a higher proportion on wrapper leaves (Liu 2004). The conditions inside lettuce heads would likely be harsher for insects, given that lettuce consumes oxygen and produce heat and carbon dioxide. In previous tests, there were incidences where only dead aphids were found in commercial lettuce heads and in artificially infested lettuce heads even though complete control of aphids were not achieved in petri dishes (unpublished data). Therefore, the complete control of N. ribisnigri in petri dishes and plastic vials means that complete control of the aphid also would probably be achieved in lettuce heads.

In the large-scale tests, temperature was raised slightly above the temperature in the small-scale tests for the same treatment durations to ensure successful control of N. ribisnigri in the large-scale tests. Sensitivity of lettuce to carbon dioxide injury is a primary concern in ULO treatments for insect control on lettuce. Previous studies showed greater sensitivity of head lettuce to carbon dioxide at the low temperature than at higher temperatures, suggesting that higher solubility of CO<sub>2</sub> in leaf tissues at lower temperatures may be the cause of the severe carbon dioxide injury (Brecht et al. 1973a). In this study, slightly raised temperatures over the normal temperature of 2°C (36°F) in the cold-chain of lettuce shipping in combination of the CO<sub>2</sub> absorption medium, soda lime, may have been beneficial in maintaining postharvest quality of lettuce. Preliminary trials showed reduction of CO<sub>2</sub> injury to head lettuce in the presence of Sodasorb (unpublished data). CO2 levels in the treatment chambers also were kept at a very low level.

Carbon dioxide injury was found to vary among different lettuce cultivars (Brecht et al. 1973c) and to increase greatly in mature compact heads (unpublished data). The six sources of commercial lettuce in this study represented a wide range of head maturity and multiple lettuce cultivars, indicated by significant differences in head weight and density among the sources (Table 4). Within each lettuce source, lettuce

heads with wide ranges of weight were evaluated. That no injury was found in ULO treated lettuce heads suggests that the ULO treatments were probably safe to most lettuce cultivars.

The results of this study showed good potential for ULO treatments in guarantine control of N. ribisnigri on lettuce without significant negative impact on lettuce quality. The ULO treatments can be carried out before lettuce is shipped in containers or during transit. In the current study, lettuce quality was evaluated 2 wk after the end of a ULO treatment, the journey for exported lettuce from the United States to Japan normally lasts ≈10-12 d. Given that there was no any sign of quality reduction at the time of quality evaluation, it is unlikely that lettuce quality would suddenly deteriorate immediately afterward. Therefore, preshipment ULO treatment is possible. However, it is still advantageous to conduct in-transit treatment and finish ULO treatment before a lettuce shipment reaches its destination port to reduce the overall length of time for lettuce to reach its markets after harvest and maximize its shelf life.

N. ribisnigri is not the only lettuce pest of quarantine significance for overseas markets. Other major lettuce insects of phytosanitary concern to overseas markets include leafminer Liriomyza langei Frick and Frankliniella occidentalis (Pergande), western flower thrips; these insects also must be controlled to remove all pest-related regulatory obstacles for export of U.S. lettuce. However, N. ribisnigri is a major lettuce pest in the United States. N. ribisnigri prefers to feed on hearts of lettuce plants and is difficult to control with contact insecticides. Therefore, the successful control of N. ribisnigri on harvested lettuce alone may reduce the risk of lettuce rejection or fumigation treatment on overseas markets and increase export of U.S. lettuce. The promising results of this study warrant commercial-scale trials to confirm the effectiveness of ULO treatment and demonstrate its feasibility for commercial use and more research efforts to develop ULO treatments for postharvest control of other pests. Given the widespread adoption of CA technology in storage and shipping of perishable commodities, ULO treatment for postharvest insect control on lettuce has good potential to be adopted by the lettuce industry.

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